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Effect of six engineered biochars on GHG emissions from two agricultural soils: A short-term incubation study

Patrick Brassard[a,b,*], Stéphane Godbout[a], Joahnn H. Palacios[a], Thomas Jeanne[a], Richard Hogue[a], Patrick Dubé[a], Lionel Limousy[c], Vijaya Raghavan[b]

[a] Research and Development Institute for the Agri-Environment (IRDA), 2700 Einstein Street, Quebec City, Quebec G1P 3W8, Canada
[b] Department of Bioresource Engineering, Macdonald Campus, McGill University, 21111 Lakeshore, Sainte-Anne-de-Bellevue, Quebec H9X 3V9, Canada
[c] Université de Haute-Alsace, Institut des Matériaux de Mulhouse (IS2M), 3 rue Alfred Werner, 28093 Mulhouse Cedex, France

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ABSTRACT

Biochar production for soil amendment was recently proposed as a tool to mitigate climate change, reducing soil greenhouse gas (GHG) emissions and sequestrating carbon (C) in soil. The aim of this research project was to test the hypothesis that only biochars with specific requirements (low H/C_{org} and O/C_{org} ratios, high C/N ratio) can reduce soil N_2O emissions without increasing CO_2 emissions in the short term. A 45-days incubation study was carried out, in which six engineered biochars made from the pyrolysis of wood, switchgrass and the solid fraction of pig manure (SFPM), were amended to two agricultural soils (loamy sand and silt loam) at a dose of 2% (w/w) in 1-liter jars. Soil moisture content was adjusted at 80% of water-filled pore space with a solution of ammonium nitrate that corresponds to 170 kg of nitrogen per hectare. N_2O and CO_2 emissions were analysed on days 2, 3, and then weekly. Soil chemical properties and bacterial richness, composition and taxonomy were analysed after the incubation period. When compared to the control soils without biochar, N_2O emissions were decreased by 42 to 90%, but only in the silt loam amended with biochars made from wood and switchgrass, these biochars having a high C/N ratio (> 30). Lower $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations were observed in these biochar treatments than in control soil. Moreover, two bacterial classes (*Deltaproteobacteria* and *Thermoleophilia*) were correlated with a decrease in N_2O emissions. For each type of biochar, those produced at the highest temperature with low O/C_{org} and H/C_{org} ratios resulted in the lowest increase in CO_2 emissions, which could indicate a higher biochar carbon stability. Overall, results of this study demonstrated that biochar can either increase or decrease soil GHG emissions depending on its properties, and that the effect can differ according to soil properties. Future long-term studies in the field in the presence of crop should be carried out in order to validate the conclusions of this study.

1. Introduction

The use of negative emission technologies for the permanent removal of carbon dioxide (CO_2) from the atmosphere was reported as a solution to limit global warming below 2 or 1.5 °C by the end of the century (UNEP, 2016), which is the objective stated in the Paris agreement in 2016. Recently, the production of biochar and its amendment to soil was identified among the most promising negative emission technologies (UNEP, 2016), having a useful negative emission potential of 0.7 Gt $C_{eq.}$ yr^{-1} (Smith, 2016). In addition, studies reported that the amendment of biochar to soil can improve soil fertility and thus increase crop yields through the improvement of soil composition, water retention, nitrification enhancement and increased nutrient uptake (He et al., 2016; Major et al., 2010; Novak et al., 2009).

Biochar is a co-product of thermochemical conversion of a biomass in an oxygen-limited environment, i.e. pyrolysis, along with syngas and the condensed bio-oil. There is a huge variability in physical and chemical properties of biochar, which depend on the feedstock and the pyrolysis operating parameters (Novak and Busscher, 2013; Y. Sun et al., 2014). Thus, not all biochars are valuable for the improvement of soil properties and as a tool to mitigate climate change.

Biochar has a high carbon (C) stability when its O/C_{org} and H/C_{org} ratios are lower than 0.2 and 0.7, respectively, and thus most of its C content ($C_{biochar}$) will be sequestered (i.e. retained) in soils for > 1000 years (Brassard et al., 2016). Moreover, many research studies demonstrated that biochars with a high C_{total}/N_{total} ratio (> 30) generally contribute to reduce soil N_2O emissions (Cayuela et al., 2014; Brassard et al., 2016). N_2O release by soils is driven by nitrification

* Corresponding author at: Research and Development Institute for the Agri-Environment (IRDA), 2700 Einstein Street, Quebec City, Quebec G1P 3W8, Canada.
E-mail address: patrick.brassard@irda.qc.ca (P. Brassard).

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(oxidation of NH_4^+ to NO_3^- via NO_2^-) under aerobic conditions, and by denitrification (reduction of NO_3^- to N_2O and N_2) under anaerobic conditions (Oertel et al., 2016). Most of the mechanisms so far identified that can be responsible for a decrease of N_2O emissions after soil biochar amendment are biotic as they involve a change in microbial abundance in the soil (Bruun et al., 2011; Harter et al., 2014; He et al., 2016; Lehmann et al., 2011). The main mechanisms include: (1) the liming effect of biochar creating an optimal environment for N_2O reductase activity (Sohi et al., 2010; L. Sun et al., 2014), (2) the reduction of the availability of NO_3^- and NH_4^+ to the microorganisms involved in nitrification and/or denitrification and producing N_2O (Kettunen and Saarnio, 2013; van Zwieten et al., 2010), (3) an enhanced soil aeration which inhibits the denitrification process (Augustenborg et al., 2012; Rogovska et al., 2011) and (4) the release of toxic/inhibitory compounds like ethylene inhibiting soil biological activity (Cayuela et al., 2014). Abiotic mechanisms have also been proposed as being involved in the mitigation of N_2O emissions in biochar-amended soils. For example, Cayuela et al. (2013) proposed that biochar could act as an electron shuttle, allowing electrons to flow more easily through the soil.

In addition, the effect of a specific biochar on soil GHG emissions and on its stability will also depend on the environmental factors, i.e. soil properties, temperature and moisture (Bai et al., 2014). However, few previous studies have evaluated the effect of different biochars in a wide range of soil conditions (Fidel et al., 2017a). For example, Ameloot et al. (2013a) reported that soil texture (especially the clay content) could have an impact on the biological response to biochar addition. Moreover, Cayuela et al. (2014) found that biochar has the greatest mitigation of N_2O in fine texture soils as compared to coarse soils.

The hypothesis of this study is that only specific biochars with low H/C_{org} (< 0.7) and O/C_{org} (< 0.2) ratios can contribute to reduce soil N_2O emissions without increasing CO_2 emissions in two types of soil in the short term. To test the hypothesis, six engineered biochars with contrasting properties produced from the pyrolysis of wood, switchgrass and the solid fraction of pig manure (SFPM), were amended in two agricultural soils, incubated over a 45-days period, and emissions of CO_2 and N_2O were analysed. In addition, the relationships between soil GHG emissions and the chemical properties, microbial diversity and abundance of the soil were studied, aiming at identifying the mechanisms involved following biochar amendment to soil.

2. Materials and methods

2.1. Biochar production and characterisation

Six engineered biochars were produced using an auger pyrolysis reactor as described by Brassard et al. (2017). Three biomasses with different physico-chemical properties were selected for the pyrolysis experiments: wood pellets made from a mixture of Black Spruce (*Picea mariana*) and Jack Pine (*Pinus banksiana*), the solid fraction of pig manure (SFPM), and switchgrass (*Panicum virgatum* L.). All biomasses were ground and sieved to a particle size between 1.0 and 3.8 mm prior to pyrolysis. Two biochars were produced from wood (W1 and W2), two from switchgrass (S1 and S2), and two from the solid fraction of pig manure (SFPM; M1 and M2). Biochars W2, S2 and M2 were produced with pyrolysis operating parameters that were chosen from a response surface methodology (RSM) by Brassard et al. (2017) and are expected to have optimal properties to maximize the $C_{biochar}$ sequestration potential (low O/C_{org} and H/C_{org} ratios). Biochars W1, S1 and M1 were produced at lower temperature and during a shorter residence time (Table 1), as determine with the RSM, to have the opposite characteristics (high O/C_{org} and H/C_{org} ratios).

The chemical properties of biochars (proximate and ultimate analysis) were analysed at the IRDA laboratory (Quebec City, QC, Canada). Moisture, volatile matter and ash contents were analysed, based on the ASTM D 1762-84 standard (ASTM, 2011). The C, hydrogen (H) and nitrogen (N) contents were evaluated by dry combustion (Leco TruSpec, St. Joseph, MI, USA). The oxygen (O) content was estimated by subtracting the C, H, N, and ash contents from 100 wt%. Inorganic C was analysed by the determination of CO_2-C content with 1 M HCl, as outlined in the ASTM D 4373-02 standard (ASTM, 2002). Organic C was calculated as total C – inorganic C. Chlorine (Cl) extraction with water and dosage by titration with silver nitrate ($AgNO_3$) was used to determine the Cl content. The specific surface area of biochar was determined by gas (CO_2) adsorption according to the Brunauer, Emmett and Teller (BET) method by using a Micromeritics ASAP2020. Prior to analysis, all samples were outgassed at 300 °C for 24 h under vacuum to remove the adsorbed species from the surface of biochars. Analysis of the biochars was carried out at 0 °C, with temperature control being achieved with an ice-water bath. Finally, the morphology of biochars was analysed using Scanning Electron Microscope—Energy Dispersive X-ray Spectroscopy (SEM-EDX - Philips XL 30 FEG) at the *Institut des Matériaux de Mulhouse* (IS2M) (Mulhouse, France).

Table 1
Pyrolysis operating parameters for the production of six biochars and their physicochemical properties.

	Unit	W1	W2	S1	S2	M1	M2
Pyrolysis parameters							
Biomass		Wood	Wood	SG[b]	SG	SFPM[c]	SFPM
Temperature	°C	516	644	459	591	526	630
Res. time[a]	s	80	101	78	104	76	94
N_2 flowrate	L min^{-1}	4.0	2.9	3.4	2.6	4.0	1.7
Products yields							
Biochar	%	26.4	18.5	26.9	18.9	46.4	34.9
Bio-oil	%	58.2	51.5	60.2	49.4	37.9	41.5
Biochar properties							
C_{total}	%	71.6	80.0	67.1	79.9	51.5	49.2
C_{org}	%	70.7	76.0	64.9	79.5	47.4	45.2
H	%	4.8	3.73	4.85	3.36	3.73	3.36
O	%	21.6	13.4	22.9	9.8	15.6	13.7
N	%	0.141	0.166	0.641	0.804	4.40	4.05
C_{total}/N	Mass ratio	508	482	105	99.4	11.7	12.1
H/C_{org}	Molar ratio	0.81	0.54	0.77	0.48	0.88	0.72
O/C_{org}	Molar ratio	0.23	0.13	0.26	0.09	0.25	0.23
$P_{soluble}$	Mg kg^{-1}	13.7	7.16	109	29.4	165	55.7
Water content	%	0.9	1.2	1.5	1.4	0.9	0.9
Ash (750 °C)	%	1.4	2.1	4.1	5.5	23.6	28.1
Organic matter	%	98.6	97.9	95.9	94.5	76.4	71.9
pH		6.8	7.6	6.4	8.8	8.6	9.3
Surface area	m^2 g^{-1}	94.2	138.1	108.7	133.2	70.9	65.1

[a] Residence time of biomass in the reaction chamber.
[b] Switchgrass.
[c] Solid fraction of pig manure.

2.2. Soil sampling and characterisation

Surface soil samples (0–15 cm) were collected in St-Lambert de Lauzon (46°36′ N and 71°10′ W) and in Deschambault (46°40′ N and 71°55′ W), which are two important agricultural regions in the province of Quebec (Canada). Based on the USDA textural soil classification, they were classified as a silt loam (20% sand, 55% silt and 25% clay) and a loamy sand (82% sand, 14% silt and 4% clay). Soils were stored at 4 °C for three weeks. Two days prior to the beginning of the incubation, soils were air-dried, ground and sieved to obtain < 2 mm fraction. Total carbon (C) and nitrogen (N) were analysed by dry combustion (Leco TruSpec, St. Joseph, MI, USA). N-NH_4 and N-NO_3 were extracted from 5 g sample in 25 g of KCl (2 M) following 1 h stirring. Water-soluble organic C (WSOC) was measured by a water extraction method. The pH was measured in water, and water content was determined by gravimetric method.

2.3. Incubation experiment

A 45-days incubation study was carried out in a plant growth chamber (Conviron, Controlled Environments Ltd., Winnipeg, Canada) in order to evaluate the effect of the six engineered biochars on the emissions of CO_2 and N_2O from soil samples. In order to mimic the environmental conditions during summer in Quebec (Canada), the growth chamber was lightened for 15 h per day while the temperature was adjusted to 22 °C during daytime and to 18 °C for the night time (9 h).

A total of 14 treatments in three replicates (two types of soil amended with six biochars, and two types of soil without biochar as control treatments) were evaluated. The biochars were added to the soil at a dose of 2% (w/w) and mixed thoroughly. Then, 747 g (d.b) of each soil and biochar mixture was added in four jars of 1 liter capacity. The bulk density (d.b.) of the silt loam and the loamy sand without biochar was adjusted to 1.20 and 1.39 g cm^{-3}, and was slightly decreased with biochar to 1.19 and 1.37 g cm^{-3}, respectively. At the beginning of the incubation period, all treatments were fertilized with a solution of NH_4NO_3 at a dose of 75.6 mg N kg^{-1}, which corresponds to 170 kg N ha^{-1}. In order to favour N_2O emissions, water was added to fill 80% of pore space (80% WFPS). In fact, according to Ussiri and Lal (2013), denitrification becomes the main source of N_2O when water content is between 70 and 80% WFPS. As the jars were kept open in the growth chamber over the incubation period, soil humidity decreased rapidly through the incubation period. Therefore, the water content was adjusted again to 80% WFPS on days 23, 37 and 44, i.e. 24 h before gas samplings.

2.4. Gas sampling and analysis

The N_2O and CO_2 fluxes from soil samples were measured at days 2, 3, 10, 17, 24, 31, 38 and 45 after the addition of the fertilizing solution. At the moments of sampling, the jars were closed tightly and gas samples were taken from the three replicates of each treatment after 30 min (t_{30}) using a 60-mL gas-tight syringe inserted through septa in a through-wall connector. Then, the gas sample was injected into air pre-evacuated 20-mL vials with septa (Gray PTFE/Black Butyl; Chromatographic Specialities Inc., Brockville, ON, Canada). In order to calculate the flux according to the linear regression scheme (Hutchinson and Mosier, 1981), additional samples were taken over the soil surface in ten random jars before they were closed, representing the initial concentration (t_0), and a gas sample was taken from a fourth jar of each treatment after 15 min (t_{15}). Samples at t_{15} were not taken from the same jars than at t_{30} in order to keep the pressure constant during the period of operation when jars were closed. On the same day, gas N_2O and CO_2 concentrations (in ppm_v) were analysed with a gas chromatography-mass spectroscopy (GC–MS; Varian 3600). Gas concentrations were converted into mg m^{-3} using the ideal gas law and the flux (mg kg^{-1} h^{-1}) was calculated by linear regression (Hutchinson and Mosier, 1981). Cumulative emissions of N_2O and CO_2 over the 45-days incubation period were calculated by linear integration of hourly fluxes starting on day two.

2.5. Analysis of soil after incubation

2.5.1. Chemical analysis

After the incubation period, the content of each jar was put in a plastic bag, mixed thoroughly, and kept refrigerated before the chemical analyses were performed on soil samples. The analyses that were carried out on soil samples before incubation were repeated on the soil and biochar mixtures after the 45-days incubation.

2.5.2. Microbial analysis

DNA extraction was done on each sample of soil and soil – biochar mixtures using commercial FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH) coupled with a FastPrep®-24 (MP Biomedicals, Solon, OH) homogenization step following the manufacturer's recommendations. The quality and the quantity of the genomic DNA obtained were evaluated by spectrophotometry on the Biophotometer (Eppendorf, Mississauga, ON, Canada) with a μCuvette® G1.0 (Eppendorf, Mississauga, ON, Canada).

The bacterial diversity was determined using high throughput sequencing and involving a library preparation step with amplification of the rDNA 16S V6-V8 region of bacteria. This was performed using the sequence specific regions described by Comeau et al. (2017) using a two-step dual-indexed PCR approach specifically designed for Illumina instruments by the *Plateforme d'analyses génomiques* (IBIS, Laval university, Quebec City, Canada).

After checking the quality of the run on MiSeq instrument, the sequences obtained were demultiplexed according to the used tag and the forward and reverse fragments were joined under QIIME v1.9.1 (Caporaso et al., 2010) using the fastqjoin tools with a minimum overlap of 50 bp. The quality of the reconstituted fragments was checked by fastqc. The paired sequences were then pooled and filtered using multiple_split_libraries_fastq.py under QIIME pipeline. For the definition of OTUs, an open reference approach was used with the reference database Greengenes 13.8 (DeSantis et al., 2006) and a grouping of OTUs within 97% of similarity. The singletons were eliminated from the bacterial OTU table.

The determination of the bacterial richness was determined by the number of OTUs observed after calculating the rarefaction curves to establish an inflection of the curves and to estimate a common number of sequences making it possible to compare the microbial richness. OTU tables were standardized to 8000 sequences by samples before computing richness diversity and comparative matrices. To compare the beta diversity of bacterial communities, comparison matrix was determined by Bray & Curtis (Beals, 1984). The multidimensional scaling (MDS) was carried out on non-Euclidean Bray & Curtis distance matrix using the “capscale” function from the Vegan package (Oksanen et al., 2007) to compare the bacterial community composition.

2.6. Statistical analysis

Analysis of variance (ANOVA) was performed using the mixed procedure of SAS (Littell et al., 2006) in order to determine significant differences in N_2O and CO_2 emissions among treatments. The fixed variables of the mixed model include the treatment, the date and the interaction date x treatment. The random variables were the replicates, the interaction treatment x replicate, and the experimental error. The date was a factor of repeated measurements with a variance covariance matrix which has been modeled to fit the correlations among the sampling on the same experimental unit. Fixed variables were all significant. The two-by-two differences among treatments were determined by the date of the sampling. The same analysis was performed for soils properties and bacterial richness. For bacterial composition, an Anosim (Clarke, 1993) analysis on Bray & Curtis distance matrix calculating an R-test was used with an estimate of the variations with 999 permutations to evaluate the effects of the DNA extraction methods on the composition of the bacterial communities.

3. Results

3.1. Biochars characterisation

Pyrolysis operating parameters, products yield and biochar properties are presented in Table 1. Biochars W2 and S2 are expected to better resist to the decomposition process as their O/C_{org} and H/C_{org} ratios are the lowest (< 0.2 and < 0.7, respectively). Since the C_{total}/N_{total} ratio of biochars produced from wood (W1 and W2) and switchgrass (S1 and S2) is higher than 30, these biochars could be better designed to reduce soil N_2O emissions (Brassard et al., 2016).

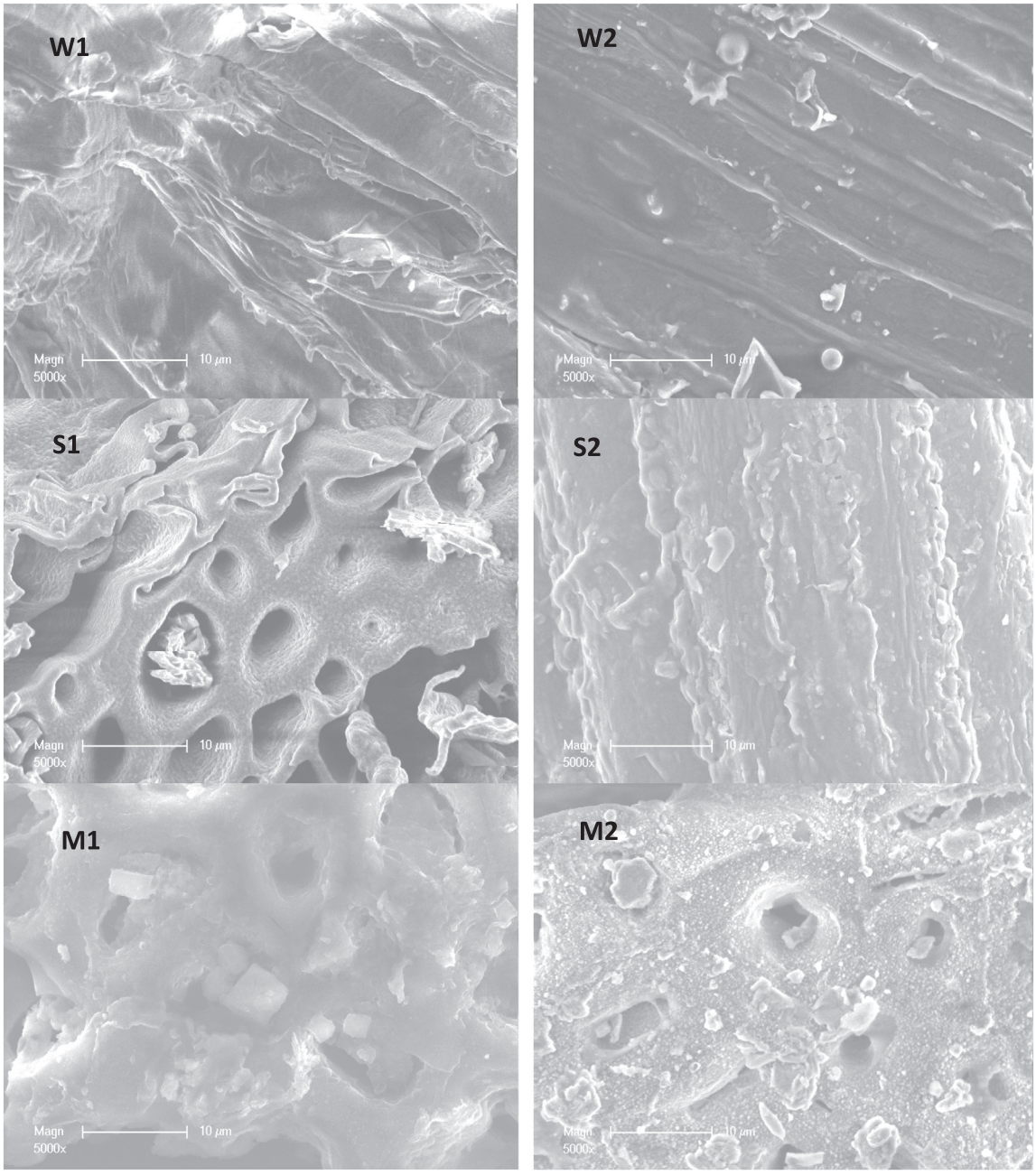

Fig. 1. SEM/EDX pictures of biochars.

Table 2
Cumulative emissions of N_2O (mg N-N_2O kg_{soil}^{-1}) and CO_2 (mg C-CO_2 kg_{soil}^{-1}) from day 2 to day 45 (mean value of three replicates ± standard error).

	N_2O emissions		CO_2 emissions	
	Loamy sand	Silt loam	Loamy sand	Silt loam
W1 (2%)	0.426 ± 0.143 *bc*	0.909 ± 0.238 *ab*	62.3 ± 7.7 *bc*	122 ± 5.5 *a*
W2 (2%)	0.730 ± 0.310 *cd*	0.740 ± 0.285 *ab*	38.7 ± 3.8 *ab*	126 ± 8.9 *a*
S1 (2%)	0.216 ± 0.085 *ab*	0.162 ± 0.014 *a*	184 ± 30.5 *d*	196 ± 17.6 *b*
S2 (2%)	0.144 ± 0.083 *ab*	0.655 ± 0.244 *ab*	81.3 ± 9.0 *c*	130 ± 4.4 *a*
M1 (2%)	1.140 ± 0.310 *d*	6.08 *d*[a]	223 ± 19.9 *e*	273 ± 13.7 *c*
M2 (2%)	0.451 ± 0.039 *bc*	2.710 ± 0.549 *c*	157 ± 9.4 *d*	213 ± 3.7 *b*
Control	0.054 ± 0.032 *a*	1.570 ± 0.186 *b*	10.2 ± 3.2 *a*	94.5 ± 7.5 *a*

Different letters in a single column indicate significant differences ($P < 0.05$).
[a] Missing data in two replicates.

The SEM pictures of biochars are presented in Fig. 1. Biochars W1 and W2 produced from wood show little apparent porosity. The same observation was made for S2 produced from switchgrass at high temperature. For sample S1, the observation was done in another part of the biochar particles. Large pores were observed due to the structure of the biomass and to pyrolysis operating parameters. Finally, M1 and M2 produced from the SFPM present also voids, and the presence of crystals at their surface was observed. EDX analysis (not shown) showed the presence of larger crystals on M2 surface corresponding to K_2SO_4 and KCl. For all biochar types (wood, switchgrass and SFPM), biochars produced at a higher temperature show the most regular patterns of voids and crevasses. Moreover, the surface area of biochars produced from wood and switchgrass is higher for biochars produced at a higher temperature (W2 and S2). This can be explained by a higher devolatilization rate of the biomass that leads to the formation of additional microporosity.

3.2. The effect of biochar on soil N_2O emissions

The N_2O emissions cumulated from day 2 to day 45, averaged from the three replicates, are presented in Table 2. For biochars produced from wood (W1 and W2) and switchgrass (S1 and S2), cumulative N_2O emissions were not significantly influenced by the pyrolysis operating parameters. However, for the biochars produced from the SFPM, emissions from soils amended with M1 produced at a lower temperature were significantly higher than those from soils amended with M2 produced at a higher temperature.

In the loamy sand, N_2O emissions were significantly increased ($P < 0.05$) in the treatments amended with biochars made from wood (W1 and W2) and SFPM (M1 and M2) as compared to the control without biochar. In the silt loam, the cumulative N_2O emissions were also significantly increased in the presence of biochars made from the SFPM (M1 and M2). At the opposite, S1 made from switchgrass at the lowest temperature contributed to the significant reduction of soil N_2O emissions by 90% ($P < 0.05$) in the mix with silt loam. A similar tendency was observed with W1, W2, and S2 even if the difference was not significant, as these biochars contributed to reduce silt loam soil N_2O emissions by 53%, 42% and 58%, respectively. These results are similar to those reported in a meta-analysis study carried out by Cayuela et al. (2015). The authors found that the average reduction in N_2O emissions in controlled laboratory studies was of 54 ± 3%.

Fig. 2 and Fig. 3 illustrate the cumulative emissions at each day of sampling. Before the irrigation on day 23, a similar trend was observed in all treatments. In fact, the hourly flux (in μg $N\text{-}N_2O\ kg^{-1}\ h^{-1}$) was the highest on day 2, decreased on day 3 and reach out very low values near zero on days 10 and 17. From day 24 to day 31, the cumulative emissions were highly increased in both soils amended with M1 and M2, which is due to the increase in the hourly flux on day 24, the next day after the soils were rewetted. For example, in the silt loam with M1, the hourly flux increased from 0.04 μg $N\text{-}N_2O\ kg^{-1}\ h^{-1}$ on day 17 to 45.33 μg $N\text{-}N_2O\ kg^{-1}\ h^{-1}$ on day 24, and it increased from 0.14 μg $N\text{-}N_2O\ kg^{-1}\ h^{-1}$ on day 17 to 21.36 μg $N\text{-}N_2O\ kg^{-1}\ h^{-1}$ on day 24 with M2. Thereafter, hourly fluxes decreased and were very low, even on the next day after the subsequent rewetting of samples on days 37 and 44.

3.3. The effect of biochar on soil CO_2 emissions

The average cumulative soil CO_2 emissions from the three replicates after the incubation period of 45 days are presented in Table 2. For biochars produced from switchgrass and the SFPM, cumulative CO_2 emissions were significantly lower ($P < 0.05$) for the biochar produced at the highest temperature (S2 and M2) as compared to those produced at a lower temperature (S1 and M1) in both soils. In the loamy sand, biochar significantly increased CO_2 emissions ($P < 0.01$) as compared to the control soil, except with W2 made from wood at elevated temperature. A significant increase in CO_2 emissions ($P < 0.05$) was also observed in the silt loam amended with biochars made from SFPM (M1 and M2) and from switchgrass at low temperature (S1), these biochars having H/C_{org} and O/C_{org} ratios higher than 0.7 and 0.2, respectively. In the same soil amended with W1, W2 and S2, CO_2 emissions were slightly increased but the difference was not significant.

Figs. 4 and 5 show a similar trend for cumulative CO_2 emissions in both soils. For all treatments, the hourly fluxes were the highest on day 2 and decreased constantly until day 17 where they stabilised, as cumulative emissions continue to increase constantly. By day 24, the hourly fluxes in the loamy sand without biochar (control) and with W1 and W2 were reduced to near zero.

3.4. The effect of biochar on soil chemical properties

The chemical analysis of the untreated soils and of all treatments after incubation are presented in Table 3 (loamy sand) and Table 4 (silt loam). The water content in the loamy sand treatments varied from 15.3 to 16.8% and from 23.2 to 26.4% in the silt loam, while it is slightly higher in biochar treatments than in the control soil. After the incubation, both control soils were acidic, with a pH of 5.4 and 4.9 in

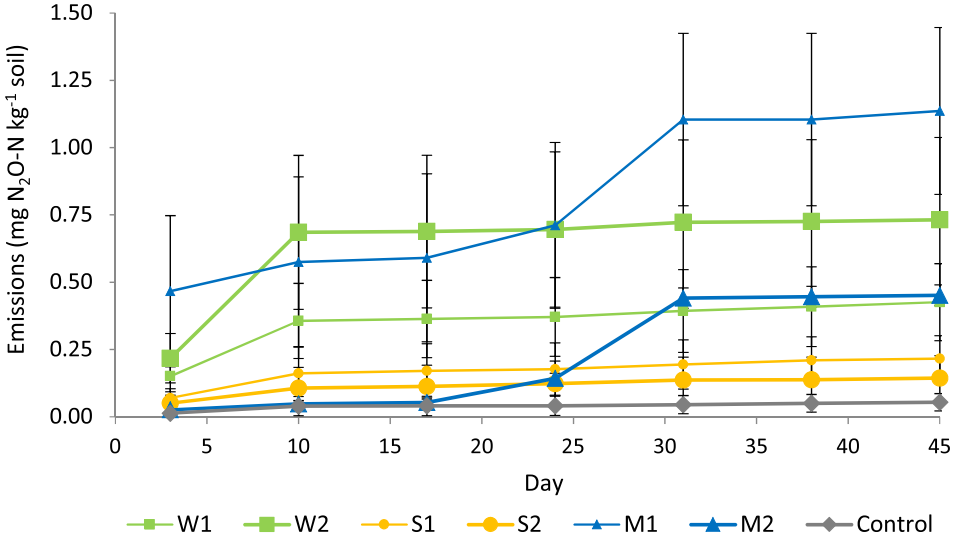

Fig. 2. Cumulative emissions of N_2O (mg $N\text{-}N_2O\ kg_{soil}^{-1}$) after the 45-days incubation period in the loamy sand – values are the mean (n = 3 replicates) ± standard errors (bars).

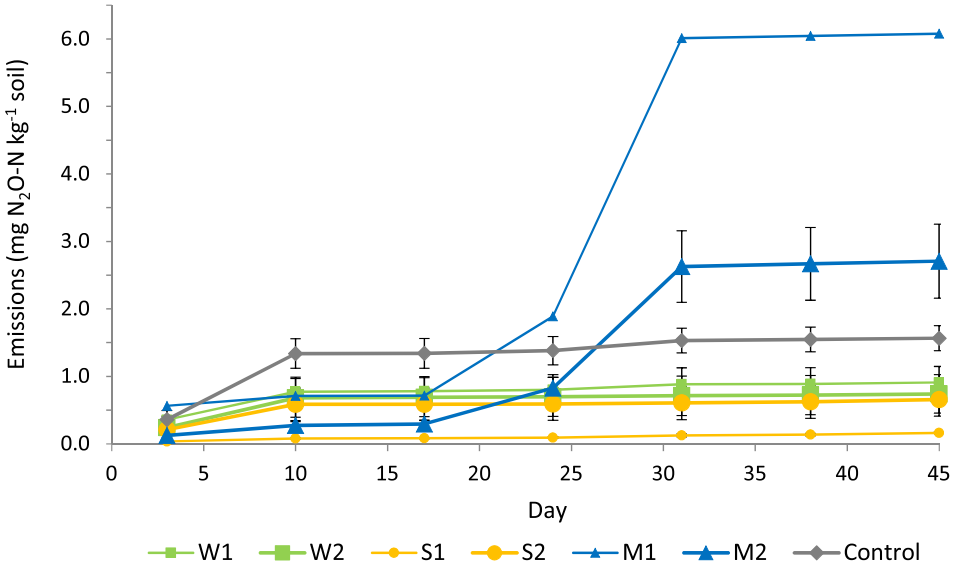

Fig. 3. Cumulative emissions of N_2O (mg N-N_2O kg_{soil}^{-1}) after the 45-days incubation period in the silt loam – values are the mean (n = 3 replicates) ± standard errors (bars).

the silt loam and the loamy sand, respectively. Biochar amendment resulted to increased pH ($P < 0.05$) for both soil types as compared to the treatment without biochar. The increase was particularly high in soil amended with biochar made from the SFPM (M1 and M2; Tables 3 and 4).

Following the incubation period, W1, W2, S1 and S2 did not have a significant impact on NH_4^+ concentration in the loamy sand; however, it was significantly increased ($P < 0.05$) with biochar made from the SFPM (M1 and M2). At the opposite, NO_3^- concentration was significantly decreased ($P < 0.05$) except with M1. In the silt loam, all biochars significantly increased ($P < 0.05$) the consumption of NH_4^+ when compared to the control soil ($P < 0.05$). Moreover, in the treatments amended with W1, W2, S1 and S2, the concentration of NO_3^- was significantly lower than in the control soil ($P < 0.05$).

C_{total} was significantly increased in all biochar treatments as compared to control after the incubation period. However, water-soluble C (WSC) concentration after incubation was not significantly different in the treatments with biochars made from wood and switchgrass (W1, W2, S1 and S2) than in the control treatments. Only biochars made from SFPM (M1 and M2) allowed a significant increase of WSC and of water-soluble organic C (WSOC) ($P < 0.05$).

3.5. Effect of biochar on the soil microbial community

3.5.1. Bacterial richness and composition

Bacterial richness index was defined with the total number of observed operational taxonomic units (OTUs) for each treatment. In both soil types, only the biochars made from the SFPM had a significant impact on the bacterial richness of soil. The α diversity analysis shows a significant decrease of the number of observed OTUs in the loamy sand amended with M1 and M2 as compared to the control soil without biochar ($P < 0.1$; Fig. 6). In the silt loam, the opposite effect was observed as the number of observed OTUs was significantly higher in the soil amended with M2 as compared to the control. In both soils, the effect of biochar produced from wood and switchgrass (W1, W2, S1 and S2) was not significant as compared to the control soils.

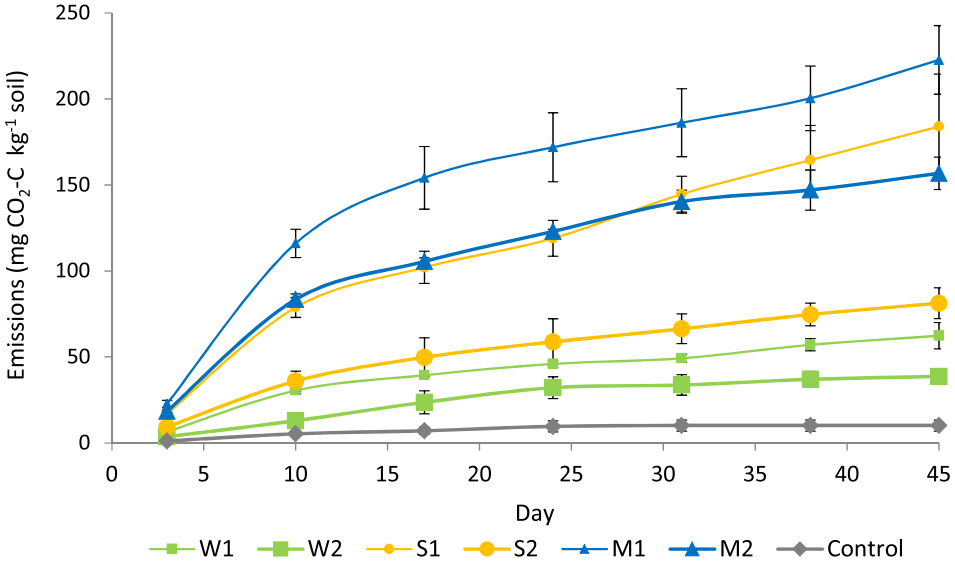

Fig. 4. Cumulative emissions of CO_2 (mg C-CO_2 kg_{soil}^{-1}) after the 45-days incubation period in the loamy sand - values are the mean (n = 3 replicates) ± standard errors (bars).

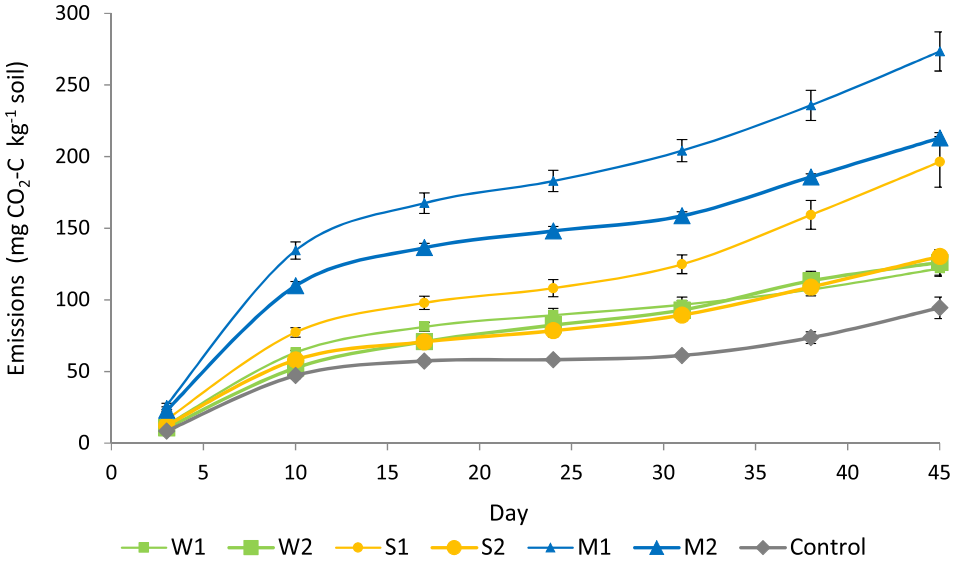

Fig. 5. Cumulative emissions of CO_2 (mg C-CO_2 kg_{soil}^{-1}) after the 45-days incubation period in the silt loam – average value of three replicates. Values are the mean (n = 3 replicates) ± standard errors (bars).

The multidimensional scaling (MDS) was carried out to compare the bacterial community composition (Fig. 7). Control soil samples as well as samples from each mix of soil-biochar can be grouped in a cluster. For each soil type, the two SFPM biochars are well separated in the cluster. The analysis of similarities that was done with the Anosim test revealed that the soil type and the SFPM biochars had a significant impact on the bacterial community composition ($R^2 = 1.00$, $P < 0.015$). In the loamy sand, the bacterial community compositions of soil mix with the biochars made from switchgrass or the SFPM were significantly different from the control ($P < 0.01$). In the silt loam, only the treatments with the biochars made from the SFPM had a significant impact on the bacterial community composition ($R^2 = 1.00$, $P < 0.015$).

3.5.2. Correlation of OTUs with N_2O and CO_2 emissions

In order to establish the relationship between bacterial composition and soil GHG emissions, the number of OTUs that are positively or negatively correlated ($P < 0.001$) with CO_2 and N_2O emissions are presented and grouped per class in Fig. 8. A negative correlation indicated that emissions are reduced with the increased OTUs of described bacterial classes. Correlations are negative for N_2O in the silt loam for six bacterial classes or 7 OTUs identified as: *Acidobacteria-6*, *Betaproteobacteria*, *Deltaproteobacteria* (2), *Gammaproteobacteria*, *Solibacteres* and *Thermoleophilia*. *Deltaproteobacteria* and *Thermoleophilia* are also correlated negatively with N_2O emissions in the loamy sand. No OTU was correlated with an increased N_2O emissions in the silt loam.

4. Discussion

4.1. Biochar effect on soil N_2O emissions: Mechanisms involved

4.1.1. Decreased soil N_2O emissions

The results of this short-term incubation study provided evidence that N_2O mitigation depends on the biochar and soil properties, and also on their impact on physico-chemical factors, microbial metabolisms and soil N cycling. Only the amendment of S1 made from switchgrass at low temperature (C/N ratio ≥ 100) in the silt loam resulted in a significant decrease ($P < 0.05$) of cumulative N_2O emissions. The lower N content in S1 as compared to S2 produced at a higher temperature could explain the significant decrease in N_2O emissions for soil amended with S1 as compared to the non-significant decrease with S2. Moreover, the results depending on the soil type are in accordance with the meta-analysis study carried out by Cayuela et al. (2014), as biochar had the greatest mitigation of N_2O emissions in the finest texture soil, which is more prone to denitrification under high water content (WFPS ≥ 80%).

One important mechanism responsible for the modification in the N cycle in biochar-amended soil, and thus for the reduced N_2O emissions, could be the limited bioavailability of electron donors and acceptors (DOC, NH_4^+ and NO_3^-) for microbial nitrification and denitrification due to (1) sorption onto biochar particles or (2) microbial immobilization as a consequence of labile C addition in the biochar (Case et al., 2012; Clough et al., 2013). A meta-analysis study demonstrated that biochar reduced soil inorganic nitrogen (SIN) by approximately

Table 3
Chemical properties of the loamy sand treatments before (t0) and after (tf) the incubation period (average value of three replicates).

	Moisture	pH	C_{total}	WSC	WSOC	N_{total}	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
	%		%	mg kg^{-1}	mg kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
Control (t0)	1.24	6.2	0.68	88.0	62.4	0.052	0.871	5.41
Control (tf)	15.3 *a*	5.4 *a*	0.611 *a*	68.6 *b*	46.6 *a*	0.047 *a*	0.602 *a*	77.4 *c*
W1 (tf)	16.3 *a*	5.5 *b*	1.170 *b*	50.9 *a*	39.6 *a*	0.056 *abc*	0.513 *a*	70.6 *b*
W2 (tf)	16.1 *a*	5.5 *b*	1.127 *b*	51.1 *a*	41.1 *a*	0.049 *ab*	0.573 *a*	70.9 *b*
S1 (tf)	16.6 *a*	5.8 *c*	1.247 *b*	63.7 *ab*	50.7 *a*	0.060 *c*	0.515 *a*	65.8 *a*
S2 (tf)	16.8 *a*	5.7 *c*	1.193 *b*	59.4 *ab*	46.4 *a*	0.058 *bc*	0.542 *a*	61.9 *ab*
M1 (tf)	16.7 *a*	6.5 *d*	1.153 *b*	145 *c*	107.6 *b*	0.095 *e*	1.090 *b*	76.5 *c*
M2 (tf)	16.7 *a*	6.9 *e*	1.051 *b*	166 *d*	107.4 *b*	0.079 *d*	0.953 *b*	66.6 *b*

Different letters in a single column indicate significant differences ($P < 0.05$).

Table 4
Chemical properties of the silt loam treatments before (t0) and after (tf) the incubation period (average value of three replicates).

	Moisture	pH	C_{total}	WSC	WSOC	N_{total}	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
	%		%	mg kg^{-1}	mg kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
Control (t0)	3.19	5.4	1.73	156.1	111.1	0.145	5.7	42.2
Control (tf)	23.5 *a*	4.9 *a*	1.68 *a*	124.8 *ab*	100.6 *a*	0.152 *ab*	2.78 *b*	153 *b*
W1 (tf)	23.8 *a*	5.0 *b*	2.92 *d*	129.0 *ab*	94.9 *a*	0.147 *a*	1.58 *a*	141 *a*
W2 (tf)	23.7 *a*	5.0 *b*	2.78 *bcd*	113.1 *a*	81.0 *a*	0.137 *a*	1.53 *a*	133 *a*
S1 (tf)	23.2 *a*	5.2 *c*	2.83 *cd*	139.0 *b*	98.4 *a*	0.158 *b*	1.27 *a*	140 *a*
S2 (tf)	25.3 *a*	5.1 *c*	2.92 *d*	140.3 *b*	92.4 *a*	0.157 *b*	1.27 *a*	134 *a*
M1 (tf)	25.6 *ab*	5.6 *d*	2.54 *b*	207.0 *c*	171.9 *b*	0.209 *c*	1.39 *a*	153 *b*
M2 (tf)	26.4 *b*	6.0 *e*	2.63 *bc*	215.7 *c*	172.0 *b*	0.215 *c*	1.54 *a*	157 *b*

Different letters in a single column indicate significant differences ($P < 0.05$).

11% ($N\text{-}NH_4^+$) and 10% ($N\text{-}NO_3^-$) in 56 studies published between 2010 and 2015 (Nguyen et al., 2017). Similar results were obtained in a study carried out by Harter et al. (2014): as DOC, NH_4^+ and NO_3^- concentrations decreased, N_2O fluxes declined. Similarly, after a 126-days incubation study, Case et al. (2012) hypothesized that the lower extractable $N\text{-}NO_3^-$ content in biochar amended soil than in the control soil could explain the N_2O suppression with increasing biochar amendment. In the present study, as N_2O emissions were significantly decreased in the silt loam with S1 and tended to decrease with W1, W2 and S2 as compared to the control soil without biochar, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations were significantly lower in these treatments ($P < 0.05$).

A first possible cause for the reduced N compounds concentration in soil is their adsorption on the biochar surface. As reported by Kettunen and Saarnio (2013) and van Zwieten et al. (2010), biochar could retain N compounds like NH_4^+ and NO_3^- due to its sorption properties, and thus affect the N cycle in soil. Moreover, Kammann et al. (2015) explained that the development of acid and basic functional groups and organo-mineral complexes on the biochar-matrix surfaces, and unconventional water-ion hydrogen-bonding to the porous surface of biochar may contribute to NO_3^- capture on/in the porous biochar matrix.

Another possible cause for reduced NH_4^+ and NO_3^- concentrations in soils amended with biochar is the immobilization of N compounds within microbial biomass. Burger and Jackson (2003) reported that C inputs in soil, for example through biochar amendment, often increase NO_3^- immobilization by stimulating the microbial activity. Many studies have already reported that biochar has an impact on microbial activity in soil (Jenkins et al., 2017; He et al., 2016). Specifically, Harter et al. (2016) indicated that biochar can affect the relative abundance and taxonomic composition of N_2O-reducing functional microbial traits in soil. Anderson et al. (2011) and Harter et al. (2014) hypothesized that decreased N_2O emissions from biochar amended soil might be caused by enhanced growth and activity of microorganisms capable of complete denitrification. The results of the present study confirm that there is a difference in bacterial richness and composition between treatments, but that the effect is specific to soil and biochar type. In fact, bacterial richness in soils amended with biochars made from wood and switchgrass is not significantly different from the control soils without biochar. However, when compared to the control soil, the number of OTUs observed in S1 treatment tends to be lower in the loamy sand, and higher in the silt loam in which the N_2O emissions were significantly decreased. Higher WSOC of the silt loam and C input by biochar could have contributed to increase bacterial richness. Considering the decrease of SIN and the increase of CO_2 emissions, there is evidence of N immobilization (Nguyen et al., 2017).

Six bacterial classes (7 OTUS) are correlated with the decreased N_2O emissions in the silt loam. Specifically, *Deltaproteobacteria* and *Thermoleophilia* are correlated negatively with N_2O emissions in both soils. It is already known that Deltaproteobacterial is a group involved

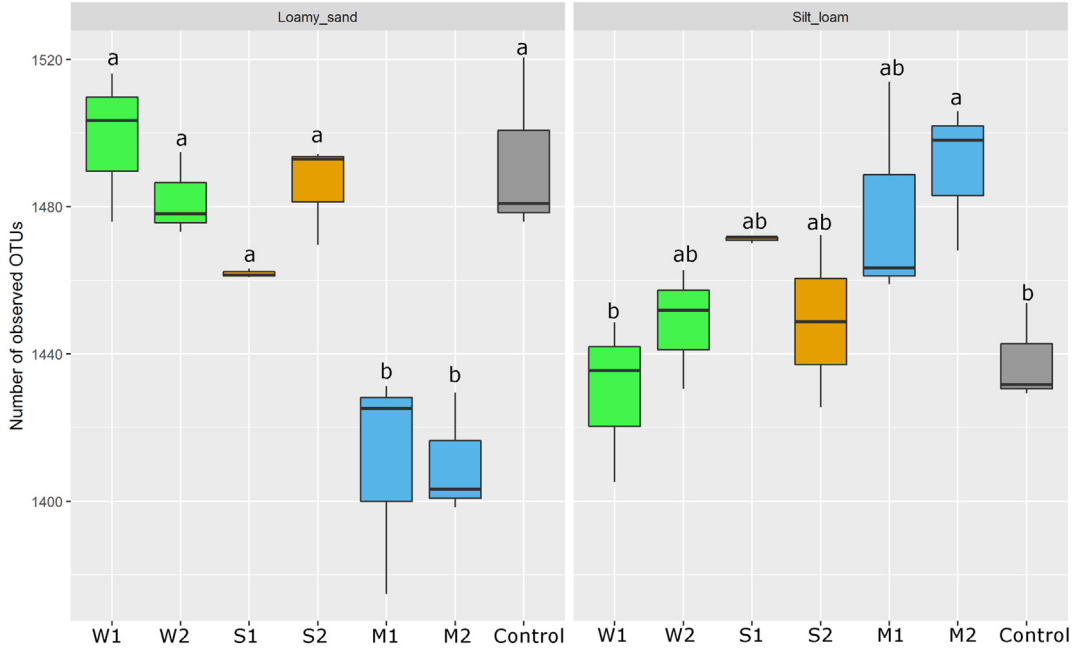

Fig. 6. Bacterial richness index defined with total number of observed OTUs for each treatment. Different letters indicate a significant difference ($P < 0.1$).

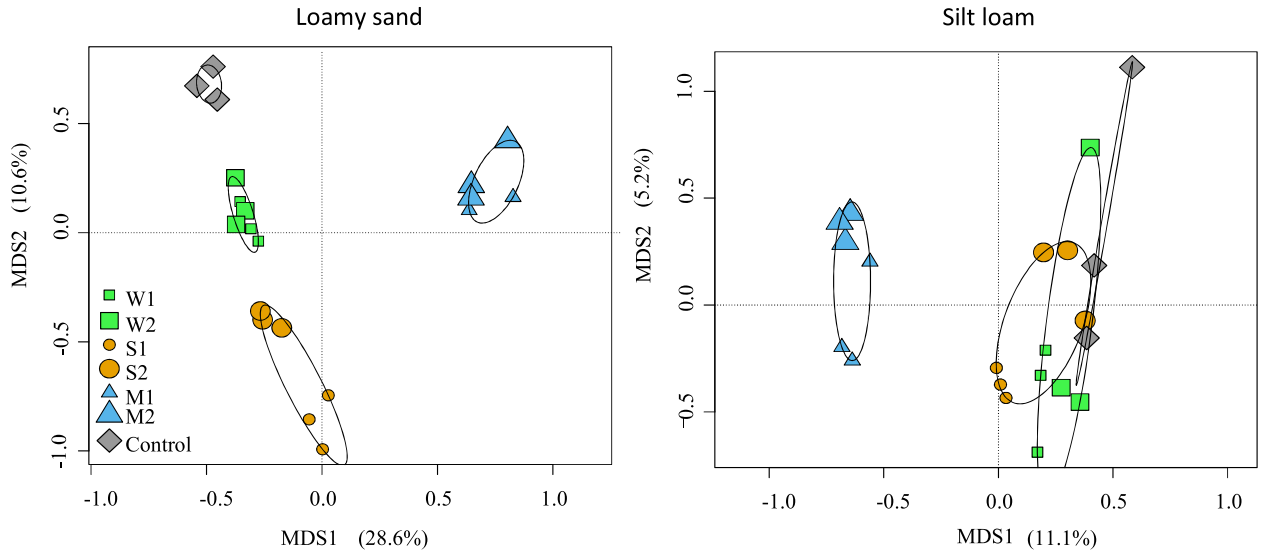

Fig. 7. Multidimensional scaling (MDS) to compare bacterial composition. The percentages on the axes are the percent of variance explained by the first (MDS1) and second (MDS2) principal components.

in the reduction of NO_3^- to NO_2^- in the denitrification cycle (Massuda et al., 2017). The *Thermoleophilia* are a proposed class of *Actinobacteria* that was created from the splitting of the *Rubrobacteridae* (Foesel et al., 2016). Actinobacteria are generally more abundant when N and labile C are added to soil (Masse et al., 2017).

According to Castaldi et al. (2011), an increased activity of N_2O-reducing bacteria due to an elevated soil pH could decrease the N_2O/N_2 ratio. In the present study, pH was increased with biochar amendment, especially with M1 and M2. The significant reduction in N_2O emissions in the silt loam was observed with S1, but not with S2. As these two soil samples have the same significant increase in pH, it indicates that the change in pH alone could not explain the reduction of N_2O emissions.

Improved soil aeration through biochar addition thus inhibiting denitrification is another mechanism proposed by Augustenborg et al. (2012) for the reduced soil N_2O emissions with biochar amendment. The SEM pictures show more voids (pores > 1 μm) in biochars obtained from the pyrolysis of switchgrass. The larger pores could provide habitat for microbial communities and contribute to maintain aerobic conditions in the soil (Lehmann and Joseph, 2009). The absence of mineral crystals at the surface of these biochars can be also outlined, because it constitutes a key parameter for bacteria adhesion on the carbon surface. Thus, the improved soil physical properties could have

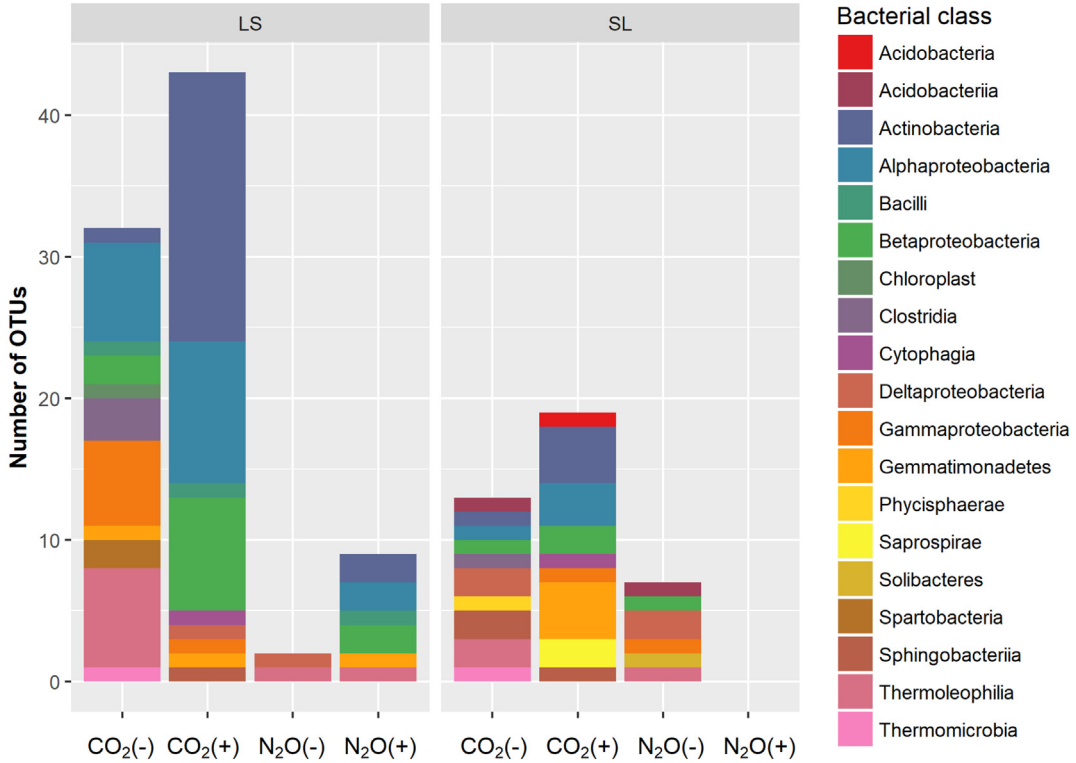

Fig. 8. Number of OTUs, grouped by bacterial classes, which are negatively (−) and positively (+) correlated with CO_2 and N_2O cumulative emissions in the loamy sand (LS) and in the silt loam (SL). No OTU was correlated with increased N_2O emissions in the silt loam.

affected the N cycle and thus could be linked to the significant decrease of N_2O emissions in the silt loam amended with switchgrass biochars, and mostly with S1. At the opposite, when mineral crystals are present at the surface of the biochars (M1 and M2 - see Fig. 1), the adhesion of bacteria may be limited at their surface (Whitlock and Smith, 2016), which can lead to higher amounts of N_2O emissions.

4.1.2. Increased soil N_2O emissions

Cumulative N_2O emissions in the control loamy sand was already low and were significantly increased with biochars made from wood and SFPM. Biochars made from the SFPM (M1 and M2), having a high N content and thus a low C_{total}/N_{total} ratio (< 30), significantly increased N_2O emissions in both soils. This could be due to significant enhanced N content is these soils as compared to the control (Tables 3 and 4). Feng and Zhu (2017) reported that soil N_2O emission was affected by the ratio of biochar to N fertilizer. The authors found a negative linear relationship between the increase in N_2O emission and soil C_{total}/IN (total carbon/inorganic nitrogen) ratio after biochar application. High C_{total}/IN ratio (> 60) was associated to the suppression of N_2O emissions, and low C_{total}/IN ratio (< 45) to the promotion of N_2O emission. In the present study, a similar conclusion can be drawn, but with higher ratios, as significant increase of N_2O emission was found in treatments with a C_{total}/IN ratio < 170. In fact, biochar produced from the pyrolysis of the SFPM is expected to stimulate N mineralization due to its high N content (Singh et al., 2012). That could explain the significant higher N_2O emissions in treatments amended with M1 and M2 as compared to the control in both soils.

The increased N_2O emissions in soil amended with biochar produced from the SFPM coincides with a change in bacterial richness and composition. Bacterial richness in soils amended with SFPM biochars is significantly different from control soil, except with M1 in the silt loam. However, the direction of this change is different depending on soil type. The loamy sand amended with M1 and M2 has significantly less observed OTUs than the control soil, as the silt loam amended with M2 has significantly more OTUs. This could be due to the higher C content in the silt loam that could feed the bacterial community. Similarly, the bacterial composition of soils amended with SFPM biochars is different from the other treatments, as the composition of M1 and M2 treatments is at one end of the clusters (Fig. 7).

4.2. Influence of biochar on soil C balance

In the present study, soil CO_2 emission was considered as an indicator of biochar short-term stability. CO_2 emissions were always higher in soils amended with biochar than in control soils. The CO_2 release was particularly high in the first 10 days, and then became nearly equal to rates in control treatments up to the end of the incubation. The fast decomposition of fresh biochar has previously been attributed to the mineralization of labile organic C due to their lower masses and simpler structures (Troy et al., 2013; Spokas et al., 2009), and to hydrolysis of inorganic C (Fidel et al., 2017b). Moreover, Ameloot et al. (2013b) reported that enhanced release of CO_2 after biochar addition to soil, which occurs mainly in the first days, may result from priming of native soil organic carbon (SOC) pools, biodegradation of biochar components from stimulation of soil organisms by biochar, or abiotic release of biochar-C. In the context of the present study, it was not possible to confirm whether biochar caused a positive or negative priming effect on SOC mineralization because biochar C was not labeled.

Despite the higher C-CO_2 emissions from the biochar treatments, C-CO_2 emitted represent a small proportion of biochar-C and does not compromise its potential to sequester C in soil (Jones et al., 2011). The additional quantity of C mineralized from the biochar treatments from day 2 to day 45 of the incubation ranged from 39 to 273 mg C-CO_2 kg_{soil}^{-1}, which represents 0.24 to 2.57% of the total C input by biochar. Indeed, priming effect is not considered, but these values are in accordance with the results from other studies in which biochar C mineralization was evaluated (Gascó et al., 2016; Luo et al., 2016; Kuzyakov et al., 2014; Steinbeiss et al., 2009; Zimmerman et al., 2011). A meta-analysis carried out by Wang et al. (2016) indicates that biochar addition can stimulate total soil CO_2 emissions by 28 to 32%. The same study revealed that the average biochar decomposition rate for studies lasting < 6 months was 0.023% per day. For example, Bruun et al. (2012) carried out an incubation study of biochar amended in a sandy loam and reported cumulative C losses of 2.9 and 5.5% for biochar produced from slow pyrolysis and fast pyrolysis of wheat straw, respectively. Other studies have shown that biochar C mineralises at a very slow rate in soils, e.g. averaged 0.1 to 3% applied biochar-C mineralized per year (Fang et al., 2015).

The cumulative CO_2 emissions depended on soil type and on the type of biochar and pyrolysis operating parameters, and particularly temperature. For biochars produced from switchgrass and the SFPM, cumulative CO_2 emissions were always lower for the biochar produced at the highest temperature, these biochars having lower H/C_{org} and O/C_{org} ratios. This is in accordance with the conclusions of other studies in which biochars produced at different temperatures where evaluated (Al-Wabel et al., 2013; Junna et al., 2014; Luo et al., 2011; L. Sun et al., 2014). Moreover, CO_2 emissions from M1 and M2 treatments were particularly high as compared to the other treatments. This could be due to the high N input by the biochars made from the SFPM. In fact, soil respiration generally increases with increasing soil N content (Oertel et al., 2016).

5. Conclusion

The results of this study demonstrated that only specific engineered biochars can reduce soil N_2O emissions without increasing CO_2 emissions in the short term. Moreover, the benefits were specific to soil properties. When compared to the control soil without biochar, N_2O emissions were significantly decreased (-90%) only in the silt loam amended with biochar made from pyrolysis of switchgrass at a low temperature and with a short solid residence time (S1), this biochar having the most important macroporosity. A similar tendency was observed with W1, W2, and S2, as these biochars with a high C/N ratio contributed to reduce soil N_2O emissions by 53%, 42% and 58%, respectively, but without significant difference. Lower NH_4^+ and NO_3^- concentrations were measured in these soil treatments. On the other hand, a change in soil microbial richness and composition as compared to the control soils was observed in soils amended with biochars produced from the SFPM and is related to increased N_2O emissions. Soil CO_2 emissions were favoured by biochar amendment, but emissions from soils amended with biochars produced at the highest temperature were lower, indicating a higher short-term stability. These biochars have lower H/C_{org} and O/C_{org} ratios. In order to validate the results of this study, long-term studies should be carried out in the field in the presence of crops.

Acknowledgments

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